

*FURTHER EXPERIMENTS ON THE INHIBITION OF THE DEVELOPMENT OF LATERAL BUDS BY GROWTH HORMONE*

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Communicated July 2, 1934

In earlier papers (Thimann and Skoog, 1933, 1934) it was shown that the inhibition of lateral buds by the terminal bud in *Vicia Faba* is due to the production of relatively large quantities of growth hormone by the latter. This substance passes basipetally through the stem, thus reaching the lateral buds, where it inhibits their growth. In these experiments it was shown that a suitable concentration of the hormone, if applied to decapitated plants, completely inhibits the development of lateral buds. The mechanism whereby a substance whose function is to promote growth may also inhibit it was interpreted in terms of the theory that in the presence of a high concentration of the growth hormone its production by a given tissue is prevented.

The present paper is concerned with two further points arising from the previous work. The first is in connection with the mechanism of the inhibition. It has been suggested by Laibach that our explanation that the growth substance inhibits bud development directly, on account of its excess concentration, is incorrect. He considers the inhibition to be only a secondary phenomenon accompanying an increased growth of the stem, which, according to him, takes place when growth substance is applied to the cut surface of the plant. Laibach's experiments are in many ways not strictly comparable with ours. He used living pollen grains as a source of growth hormone, and his paper implies that the plants were decapitated so close to the base that only the cotyledonary buds could develop. Under these circumstances he found that when pollen grains were applied to the decapitated stump a marked increase in growth, particularly in thickness, took place, and the outgrowth of the cotyledonary buds was retarded. In our experiments, on the other hand, a preparation of growth hormone obtained from *Rhizopus suinus*, and of known purity (about 5%) was used. The plants were grown until 3 or 4 leaves had developed; the terminal bud and youngest leaf were then removed and the growth of the lower lateral buds (Nos. 1 and 2 in Fig. 1) was determined. Given amounts of growth substance in agar, or of plain agar, were applied to the cut surface of the stem at regular intervals. Under these conditions cotyledonary buds did not develop. In all our experiments it was observed that (1) there was no increase in *thickness* of the stem in plants to which growth hormone was applied, and (2) as is shown in table 7 of the earlier paper (1934),

even when the concentration of applied growth hormone was sufficient to cause complete inhibition there was no increase in *length* of the stem over the controls. The reason for this, as was shown with defoliated plants, is that the amount of residual growth substance in the plant, and the amount synthesized by the green parts, is enough to supply all that can be utilized

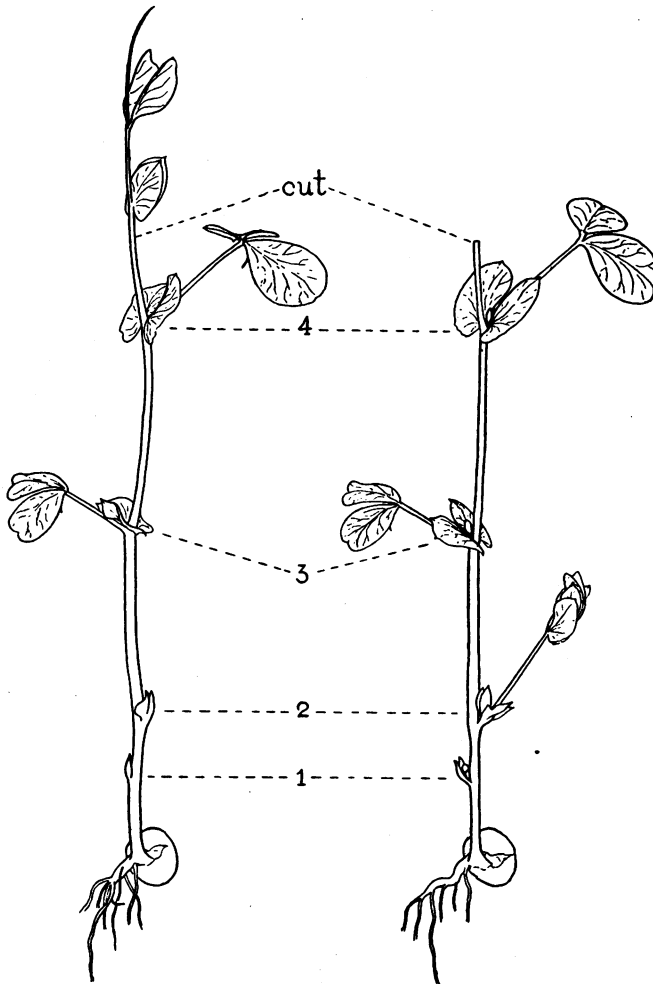


FIGURE 1

in stem elongation. Laibach's argument that the growth hormone concentration used in the above experiments was insufficient to show the growth-stimulating effect is therefore invalid. On the contrary, so far as growth-promotion is concerned only small amounts are necessary, and these are already present; for inhibition, the concentration of applied hor-

mone, like the concentration diffusing from the terminal bud, must be relatively high. As was concluded earlier, therefore, the inhibition of lateral buds cannot possibly be ascribed to any stimulation of growth.

The second point has to do with the possibility that the inhibition is due not to the growth hormone itself but to a special inhibitor present in our preparations. This seems improbable, since the inhibitor, if it were present in the purified preparation, must have been produced, together with the growth substance, both in the mould culture and in the tip of the experimental plants. Nevertheless the question of the existence of a special inhibitor must be settled before the explanation that the inhibition is due to the growth substance itself can be definitely accepted. If it could be shown that the completely pure growth substance inhibits as actively as the relatively impure preparation this point would be proved. Through the generosity of Professor Kögl we have now been able to test his crystalline preparations from urine and other sources (Kögl, Haagen-Smit and Erxleben, 1933) for their inhibiting activity. The present experiments show that these preparations are at least as active in inhibiting development of lateral buds as the solutions previously used.

*Material and Methods.*—It was found by Snow (1929) that the behavior of *Pisum sativum* with regard to bud development is very similar to that of *Vicia Faba*. Since peas are somewhat more convenient we have used them exclusively. The strain "Alaska," a pure line, was grown in the greenhouse until three leaves had developed and then decapitated so that two leaves

TABLE 1  
INHIBITION BY CRYSTALLINE HETERO-AUXIN

*Pisum*: 20 plants, 2 weeks old; no leaves present; applications every eight hours for 6 days; mean stem length at start approx. equal; measurements of Bud No. 2 only.

	DAY 0	LENGTH OF BUD IN MM. DAY 4	DAY 6	LENGTH OF STEM IN CM. DAY 6
Decapitated; water applied	<1.5	5.2 $\pm$ 0.8	9.0 $\pm$ 2.0	11.2 $\pm$ 0.3
Decapitated; 0.05 cc. of Hetero-auxin, 7000 units per cc. applied	<1.5	<1.5	<1.5	11.6 $\pm$ 0.3

remained (see Fig. 1). In the experiment of table 1 younger plants were used, in which only one leaf had developed, and this was removed with the terminal bud. The bud developing in the stipule below the oldest leaf (Bud No. 2) was principally observed. Small paraffin cups were moulded onto the cut surface of the stem and an aqueous solution of growth substance introduced into the cup. The cups were refilled every eight hours. The experiments were carried out in a humidity high enough to prevent rapid evaporation. This technique was found as satisfactory for our purposes as the application of agar blocks, and less time-consuming.

*Results.*—A preliminary experiment, using agar blocks containing Auxin A (auxentriolic acid) Auxin B (auxenolonic acid), Hetero-auxin, and the *Rhizopus* preparation Br.F.\*, similar to the preparation used in our previous experiments, gave the following results: Auxin A, which had lost almost all its growth-promoting activity when received, produced no inhibition; Auxin B and Hetero-auxin, at a concentration of about 1000 units per cc., gave about the same inhibition as Br.F. at the same concentration.

The next experiment (table 1) proves that the crystalline compound is capable of causing complete inhibition without any increase of stem length. The buds less than 1.5 mm. long were not measured, since, in order to avoid any possible damage to them, the stipules were not removed.

Inhibition by the crystalline substance is thus complete, and this being proved, it only remains to show that for a given concentration in growth-promoting units, the crystalline compounds produce as great an inhibition as the impure preparation. Hence with the remaining available material the activities were standardized with *Avena* and adjusted to concentrations of 1000, 3000 and 5000 units per cc., and compared with Br.F. at the same concentrations. The results are given in table 2. At the start all buds were less than 1.5 mm. long. The experiments had to be discontinued after 5 days, and in three solutions after 4 days, owing to shortage of material. In this experiment buds other than No. 2 also developed to some extent, and their measurements are included in the table; it may be seen that they develop in the same ratio as the others, except that Bud No. 4 is relatively little affected.

TABLE 2

## COMPARISON OF HORMONE PREPARATIONS AT DIFFERENT CONCENTRATIONS

Plants grown in light; all buds less than 1.7 mm. long at start.

BUD DAY NO.	DECAP. CONTROLS	LENGTH OF NUMBERED BUD IN MILLIMETERS						HETERO-AUXIN			INTACT CONTROLS
		AUXIN B 3000	AUXIN B 5000	BR.F.(RHIZOPUS)			1000	3000	5000		
4 2	7.8	3.3	2.6	4.4	4.3	3.2	2.6	2.3	2.4	1.7	
	±0.6	±0.3	±0.2	±0.6	±0.8	±0.8	±0.1	±0.1	±0.3	±0.1	
5 2	14.0	4.8	4.4*	7.5	8.1	6.0	3.7	3.8*	4.5*	1.7	
	±0.6	±0.8	±0.6	±1.5	±1.8	±1.5	±0.8	±0.3	±0.5	±0.1	
5 1	2.6	1.1	1.2	1.2	1.2	1.2	1.0	1.8	1.0	no develop- ment	
5 3	6.4	3.7	2.6	4.3	3.6	3.4	2.9	2.8	2.6	no develop- ment	
	±1.2	±0.4	±0.2	±0.9	±0.3	±1.0	±0.4	±0.3	±0.5		
5 4	2.6	2.0	1.8	2.4	1.2	1.2	1.9	1.8	1.4	no develop- ment	
No. of plants	15	10	7	9	7	7	11	5	5	15	

\* Application of hormone stopped at middle of 4th day.

On account of the small number of plants used the results do not show a smooth variation with concentration. However, it is clear that the two

crystalline compounds are, unit for unit of growth substance, at least as active as the *Rhizopus* preparation Br.F. The table further shows that the Hetero-auxin produces a more complete inhibition than the other two substances. This is in accordance with the fact that while the latter preparations steadily decrease in activity on keeping, Hetero-auxin remains constant, and therefore retains its full activity throughout the experiment. Further, Hetero-auxin is much less readily attacked by oxidizing agents than the other compounds and therefore will be less inactivated in the plant. That this loss in activity may be considerable has been shown by inactivation with crushed plant tissue (Thimann, 1934).

On account of the possibility that the plants might be permanently injured by the application of high concentrations of growth hormone, the buds were re-measured 9 days after the close of this experiment. As a rule Bud No. 2 at first develops the most rapidly, but in a few cases one of the other buds may subsequently overtake it. When this happens, the more rapidly growing bud may completely stop the growth of Bud No. 2, as has been previously discussed (Thimann and Skoog, 1934). In order to determine the total amount of bud elongation, therefore, it was necessary to add together the final lengths of buds 1, 2, 3 and 4. The mean total bud lengths in mm. on the 14th day after decapitation were, for the various concentrations of growth hormone, as follows:

Decapitated controls	Auxin B		Hetero-auxin			Br. F.			Intact controls
	3000	5000	1000	3000	5000	1000	3000	5000	
184	174	169	117	155	120	156	194	185	<4

The figures show that the inhibited buds grow in a normal manner after the application of hormone has ceased. The buds are now equal in length to those on the decapitated controls in all but the Hetero-auxin series. The latter are still somewhat behind the others; the buds are, however, rapidly growing, and the difference is to be ascribed to the greater stability of the Hetero-auxin, as discussed above.

*Conclusions.*—The inhibition of lateral buds brought about by the application of growth substance after decapitation is not due to a stimulation of the growth of the experimental plants, since inhibition is complete without any accompanying increase in stem length or thickness. The application of two different crystalline preparations of growth substance, auxin B and Hetero-auxin, to decapitated *Pisum* shows these to be at least as active in causing inhibition as the impure preparation from *Rhizopus*, when used in the same growth substance concentrations. A third crystalline compound, auxin A, which had lost most of its growth-promoting activity produced no inhibition. The inhibition produced by the application of the growth hormone is not accompanied by any injury to the plant.

\* Activity 12,000 units per mg. =  $2.4 \cdot 10^6$  plant units per mg. = 5% pure growth substance.

Kögl, F., Haagen-Smit, A. J., and Erxleben, H., *Z. Physiol. Chem.*, **216**, 31 (1933).

Laibach, F., *Ber. d. Deutschen Bot. Ges.*, **51**, 336 (1933).

Snow, R., *New Phytologist*, **28**, 345 (1929).

Thimann, K. V., *J. Gen. Physiol.* (in press).

Thimann, K. V., and Skoog, F., these PROCEEDINGS, **19**, 714 (1933).

Thimann, K. V., and Skoog, F., *Proc. Roy. Soc.*, **B114**, 317 (1934).

## THE POTENTIAL OF A POSITIVE MASS AND THE WEIGHT FUNCTION OF WIENER

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Communicated July 3, 1934

*The Potential of a Positive Mass.*—Let  $\mu(e)$  be a completely additive non-negative function of sets measurable Borel in three dimensions. The integral

$$u(M) = \int_W \frac{1}{MP} d\mu(e_p),$$

where the region of integration is all of space, is defined as  $\lim_{n \rightarrow \infty} \int_W \varphi_n(M, P) d\mu(e_p)$ ;  $\varphi_n(M, P) = 1/MP$  if  $1/MP \leq n$ , and is equal to  $n$  otherwise. The function  $u(M)$  is called the potential of the mass distribution  $\mu(e)$ .

In the sequel it will be assumed that  $\mu(e) = 0$  for all  $e$  measurable Borel outside some sufficiently large sphere, and that  $u(M)$  is bounded.

A point  $P$  will belong to the set  $F$  if and only if  $\mu(e) > 0$  for every sphere  $\sigma$  with center  $P$ ;  $e$  is the set of points on and interior to  $\sigma$ . Obviously  $F$  is closed. We should then have

$$u(M) = \int_F \frac{1}{MP} d\mu(e_p).$$

This follows from the fact that for a fixed  $M$  the integral is an additive function of sets.<sup>1</sup>

The volume average

$$A_n(P_0) = \frac{3n^3}{4\pi} \int u(P) dv$$

of  $u(M)$ , where the integral is taken over the volume of the sphere with center  $P_0$ , and radius  $1/n$ , is continuous and for fixed  $P$  an increasing function of  $n$ ; furthermore  $\lim_{n \rightarrow \infty} A_n(P) = u(P)$ .<sup>2</sup>